Using Manganese-Enhanced MRI (MEMRI) to detect the order of neuronal connections in the olfactory pathway at the level of specific layers

D-Y. Chen[1], S. J. Dodd[2], D. R. Glen[2], Z. S. Saad[2], and A. P. Koretsky[2]
[1]NINDS, National Institutes of Health, Bethesda, Maryland, United States, [2]National Institutes of Health

Introduction: It has been shown that manganese-enhanced MRI (MEMRI) can be used to perform anterograde neuronal tract tracing in a number of neural systems, including the olfactory pathway [1,2]. Furthermore, MEMRI can be used to visualize laminar structures in the olfactory pathway and cortex *in vivo*. Recently the layer-specific input to somatosensory cortex from thalamus and contralateral cortex could be observed if scanning occurred at the proper time intervals after Mn^{2+} administration [3]. In the present study the dynamic changes of manganese enhancement has been used to detect of the order of inputs at the level of specific layers in the rodent olfactory system. Tracing to the olfactory bulb (OB), anterior olfactory neuron (AON), piriform cortex (PCx) and orbitofrontal cortex (OFC) was studied after $MnCl_2$ was infused to nostrils or the olfactory bulb of rats. The order of estimated arrival latency followed the known anatomical connections.

Methods: To examine Mn^{2+} enhancement at high spatial resolution with good sensitivity, a Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence was used. All images were acquired with an 11.7T/31cm horizontal bore magnet (Magnex, Abingdon, UK), interfaced to an AVANCE III console (Bruker, Billerica, MA). The 3D 100-µm isotropic MP-RAGE imaging was performed with the following parameters: FOV= 2.56x2.56x1.28 cm, matrix 256x256x128, TR= 4000 ms, Echo TR/TE = 15/5 ms, TI= 1000 ms, number of segments= 4, Averages= 4. The total acquisition time of each averaged set of volumes was 136 min. A total of 14 male SD rats were used in this study. $MnCl_2$ (500 mM, 20 µl) were infused to bilateral nostrils of rats (n=8), and another group of rats (n=6) received $MnCl_2$ (100 mM, 0.1 µl) injections into their olfactory bulb. Both groups were anesthetized by isoflurane. In addition to a pre-infusion scan, post-infusion images of the same animal were acquired at several time points up to 48h after $MnCl_2$ infusion. Images of the same rat at these different time points were aligned with the AFNI software package using a local Pearson correlation method [4] that was modified for aligning image gradient across time. In each animal, the time course of the normalized intensity in each layer and region was modeled at an ROI level and at a voxel-wise level. At the ROI level, the mean across time of an ROI was modeled with a sigmoidal function: $y = 1/(1+\exp(a*(b+x)))$. The latency to the half-maximum of the fitted curve ($T_{1/2}$) was used to indicate the estimated manganese arrival latency to the specified area. At the voxel level, each voxel was modeled across time using a non-linear fitting program (3dNLfim) with a simple exponential model. A $T_{1/2}$ was automatically calculated at a voxel level over the entire volume.

Results: Mn^{2+} enhancement was detected along the olfactory pathway at different time points. In general, Mn^{2+} reached the OB first, then AON, PCx and OFC. In addition, the fiber tract and superficial layer were labeled earlier than the deep layer. Figure 1 shows that the estimated arrival latency of the lateral olfactory tract (lo) and the superficial layer of PCx (layer 2) are significantly shorter than the deep layer (layer 3). This result is consistent with histological evidence [5] that the superficial cell layer of PCx receives afferents from the olfactory bulb. Figure 2 shows that the estimated arrival latency of the superficial layer of OFC was significantly shorter than the deep layer. Similar results also could be found in rats that received manganese injections into their olfactory bulb, except that the estimated arrival latency that was shorter than those that received the nostril injections. In addition to the analysis with manually drawn ROIs, a voxel-wise curve-fitting approach was also used to map the estimated arrival latency without subjective bias (Figure 3). The mapping results showed similar layer structure, consistent with the ROI analysis.

Conclusions: MEMRI can distinguish laminar structure in the olfactory system and trace the layer-specific inputs from peripheral to cortex. Precise alignment of images across time allows the estimation of arrival latency of manganese into specific layers. The estimated arrival latency shows that manganese arrives at superficial layers of the olfactory cortex earlier than deep layers, which is consistent with known connections. Furthermore, the results indicate that the input from olfactory cortex is into the superficial layer of orbitofrontal cortex, a connection that has not yet been well studied.

References: [1] Pautler et al, MRM 40:740-748 (1998). [2] Chuang et al, MRM 55:604-611 (2006). [3] Tucciarone et al Neruoimage 44, 923-931 (2009) [4] Saad et al, NeuroImage, 44: 839-848. [5] Price, J. Comp. Neur. 150: 87-108 (1973).

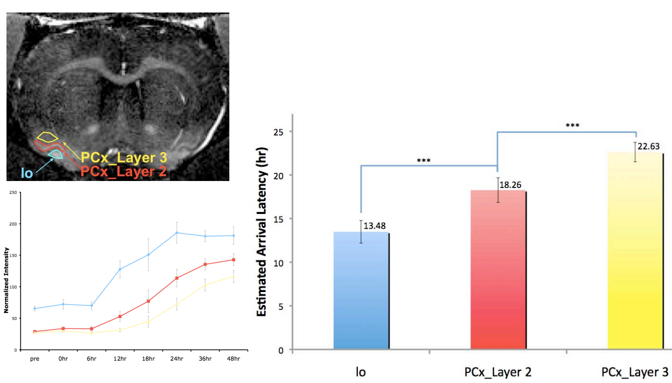

Fig 1. Time course of normalized intensity in both layers of piriform cortex and the lateral olfactory tract (left panel). Estimated arrival latency among layers was significantly different (right panel).

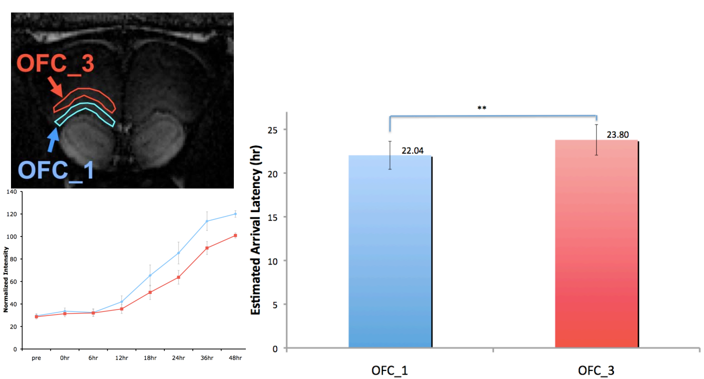

Fig 2. Time course of normalized intensity in both layers of the orbitofrontal cortex (left panel). The estimated arrival latency of the superficial layer of OFC was significantly shorter than the deep layer (right panel).

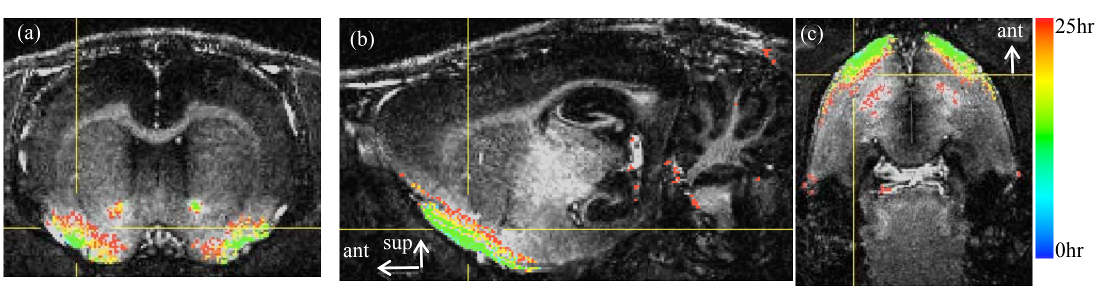

Fig 3. The arrival latency estimated by voxel-wise fitting approach reveals the layers in the piriform cortex. (a) coronal view, (b) sagittal view, and (c) transverse view.